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Investigation of phytochemical composition of new plant collection with antidiabetic activity (notice II - secondary metabolites)

Alona Savych, Svitlana Marchyshyn and Sofiia Nakonechna

Abstract
Studied the chemical composition of antidiabetic plant collection. Is was revealed and defined quantitative content of compounds of secondary synthesis: flavonoids, hydroxycinnamic acids, tannins, essential oils. The chlorogenic acid, rosemary acid, ferulic acid, coffee acid, p-coumarin acid; flavonoids– apigenin, rutin, luteolin, hyperoside, isoquercetin, quercetin; components of condensed tannins – galocatechine, epigalocatechine, catechine, epicatechine, catechine galat, epicatechine galat; free tannins – gallic and ellagic acid, and coumarin have been identified using the method of high performance liquid chromatography (HPLC). Established the quantitative content of selected individual substances. It was investigated the composition of essential oil in antidiabetic plant collection, was identified 29 substances, the main ones are alantolaktone, isoalantolactone, cariophilenoxyde, tricosane, trans-epoxyllinalool.

Keywords: Diabetes mellitus, antidiabetic plant collection, tannins, flavonoids, hydroxycinnamic acid, coumarin, essential oils

1. Introduction
Diabetes mellitus is a state of chronic hyperglycemia, resulting from absolute or relative lack of insulin caused by the influence of various exogenous, immune, endocrine and genetic factors, or their combination. In the structure of endocrine diseases, its share reached 70%, which becomes threatening proportions [1].
Diabetes treatment requires to follow certain principles that will ensure sufficient quality of life and favorable prognosis.
Since diabetes affects all types of metabolism, blood vessels and internal organs, the administration of hypoglycemic therapy may be insufficient, so it is advisable to use drugs with a systemic effect on the body, including herbs because that have a wide range of pharmacological actions due to the presence of biologically active substances of different genesis and diverse effects on the body. This enables conducting as the prevention as well treatment of diabetes and prevent serious complications: micro-and macroangiopathy [2].
Plants in the course of their life synthesize a number of low molecular organic compounds which are called secondary metabolites. This group of substances has found its practical value in traditional and folk medicine, due to the high physiological and pharmacological activity. Phenolic compounds (flavonoids, hydroxycinnamic acid, coumarin, tannins, xanthones, lignans), essential oils belong to the substances of secondary synthesis [3].
The aim of our work was to determine the qualitative composition and quantitative contents of secondary synthesis substances in antidiabetic plant collection, which includes herbs traditionally used in folk medicine for treatment diabetes: *Equiseti arvensis herba, Sambuci flores, Inulae rhizomata et radices, Hyperici herba, Tiliae flores, Polygoni avicularis herba, Myrtilli folium, Urticae folia* [4].

2. Materials and methods
It was conducted the qualitative detection and was established quantitative content of phenolic compounds and essential oils in antidiabetic plant collection. For detection of tannins was used the solution of iron (III) ammonium sulfate (the appearance of blue-black color indicative of the presence of tannins); the determination of the quantitative content of tannins was carried by permanganometry [5].
Detection of flavonoids and hydroxycinnamic acids was carried by chromatographic method. Conducted the thin layer chromatography (TLC) using plates "Silufol UV 254" 15x15 cm

company "Kavalier" (Czech Republic) in the solvent n-butanol - acetic acid - purified water (4:1:2). Chemical substances detected before and after processing the chromatogram by ammonia vapors and 3% solution of iron (III) chloride in color in daylight and by their fluorescence in filtered UV light [5, 6].

The content of amounts of flavonoids and hydroxycinnamic acids was determined by spectrophotometric method on a spectrophotometer Cary 50, by measuring the optical density at a wavelength of 415 nm and 327 respectively [6-8].

For separation the amounts of phenolic compounds into individual components was used high performance liquid chromatography (HPLC) by chromatograph Agilent 1200 3 D LC System Technologies (USA), which is equipped with a flow vacuum degasser G1322A, four-pump gradient low pressure G13111A, autosampler (auto-injector) G1329A, thermostat columns G 1316A, refractometric detector G1362A.

To determine the phenolic compounds in the studied objects was carried out reversed-phase chromatography using a chromatographic column Supelco Discovery C18 measure 250×4.6 mm with sorbent silica gel modified okta decile group, which has a diameter of 5 mm.

For the separation of phenolic compounds used the following parameters of chromatographic analysis: gradient elution, the mobile phase–bidistilled water which acidulated 0.005 n solution of phosphoric acid ("A") and acetonitrile ("B") – analysis of hydroxycinnamic acids, flavonoids, coumarins; 0.1% solution of trifluoroacetic acid, 5% solution of acetonitrile (A) and 0.1% acetonitrile solution of trifluoroacetic acid (B) – analysis of components of tannins. Scan time – 0.6 seconds, the detection range – 190-400 nm, wavelength of detection in ultraviolet spectra – 320 and 330 nm (hydroxycinnamic acid, coumarin, flavonoids) and 280 and 255 nm (components tannins). Total analysis time – 50 and 40 minutes [9-11].

Regime of chromatography: maximum feed rate of the mobile phase – 0.7 ml/min, eluent operating pressure – 100-120 bar (10000-12000 kPa); temperature of thermostat column – 25 °C; volume administered tests - 5-20 ml, chromatography time - 50 minutes.

Gradient elution regime (Table. 1). The rate of the mobile phase and a working pressure of 0.8 ml/min, 156.105 Pa (flavonoids, hydroxycinnamic acid, coumarin) i 0.1 ml/min, 400 bar (components tannins), temperature of thermostat column 250 °C.

Table 1: Parameters of gradient elution regime

Flavonoids, coumarin							
Time, min	0	30	33	38	40	41	49-60
Eluent B, %	12	25	25	30	40	80	12
Hydroxycinnamic acid and hydroxy-coumarin							
Time, min	0	8	15	30	40	41	43-50
Eluent B, %	5	8	10	20	40	75	5
Fragments of tannins							
Time, min	0	8	10	15	20	25	28	29-40
Eluent B, %	100	12	12	25	25	75	75	100

For analysis the plant raw material was crushed and took 1 g (exact sample), was placed in a round bottom flask 100 ml, added 50 ml of 60% methanol, flask were attached tor everse refrigerator and heated in a boiling water bath for 15 minutes while stirring. Then the contents of the flask sonicated for 10 minutes, filtered and quantitatively transferred to dimensional flask with a capacity 100 ml and adjusted volume by 60% solution of methanol to the mark [11].

Phenolic compounds identified by the results and retention times comparison the UV spectra of standard samples. The calculation of concentrations was carried out for calibration characteristic.

The research a component of essential oil of the studied materials was performed by Agilent Technology 6890N chromatograph with mass spectrometric detector 5973N. Essential oil components were identified by results of comparison to the data library mass spectra NIST02 during the chromatography of mass spectra of chemicals. The indices of obtaining component calculated on the results of analyzes of essential oil with the addition a mixture of normal alkanes (C10-C18) [12]. The study was conducted at the National Institute of Vine and Wine "Magarach" in Ukrainian Academy of Agrarian Sciences together with lead engineer BO Vinogradov.

3. Results and Discussion

It was found out condensed tannins in antidiabetic plant collection by means of the identification reactions. The quantitative content of tannins in antidiabetic plant collection was (6.86±0.81) % in terms of dry materials.

By TLC have been identified rutin, quercetin, kaempferol, hyperoside, apigenin in the antidiabetic plant collection, which acquire in UV light from yellow to brown varying intensity, which increased under the influence of ammonia vapors. The results of spectrophotometric study showed that the content of flavonoids in the antidiabetic plant collection was (2.30±0.04) % in terms of rutin.

It is known that one of the important features of flavonoids have antioxidant effects. Their phenolic structure allows the molecule flavonoids interacting with free radicals, reducing the intensity of lipid peroxidation. Antioxidant effect of flavonoids increases resistance to various adverse environmental factors. Antioxidant properties of flavonoids contribute their hypocholesterolemic and anti-sclerotic effect, membrane-stabilizing properties. They detect antiallergic, antidiabetic, diuretic, antispasmodic, hypotensive effect, expand coronary vessels, increase myocardial contractile properties [13-15].

Hydroxycinnamic acids also have pronounced pharmacological activity: anti-inflammatory, hepatoprotective (coffee acid, ferulic acid) and immunotropic (coffee acid) cholagogue, antimicrobial, antifungal, radioprotective (ferulic acid) effect [16-18].

By TLC in antidiabetic plant collection were found chlorogenic, coffee, ferulic and p- coumarin acid. Spectrophotometer study showed that the total content of hydroxycinnamic compounds in the test assembly was (3.10±0.07) %, in terms of chlorogenic acid.

As a result of HPLC analysis in antidiabetic plant collection was identified and was defined quantitative content of individual phenolic compounds, hydroxycinnamic acids: chlorogenic, rosemary, coffee, ferulic and p- coumarin acid; flavonoids: apigenin, rutin, luteolin, hyperoside, isoquercetin, quercetin and condensed components: galocatechine, epigalocatechine, catechine, epicatechine, catechine galat, epicatechine galat; free tannins: gallic and ellagic acid, and coumarin (Figure 1- 6; Table. 2).

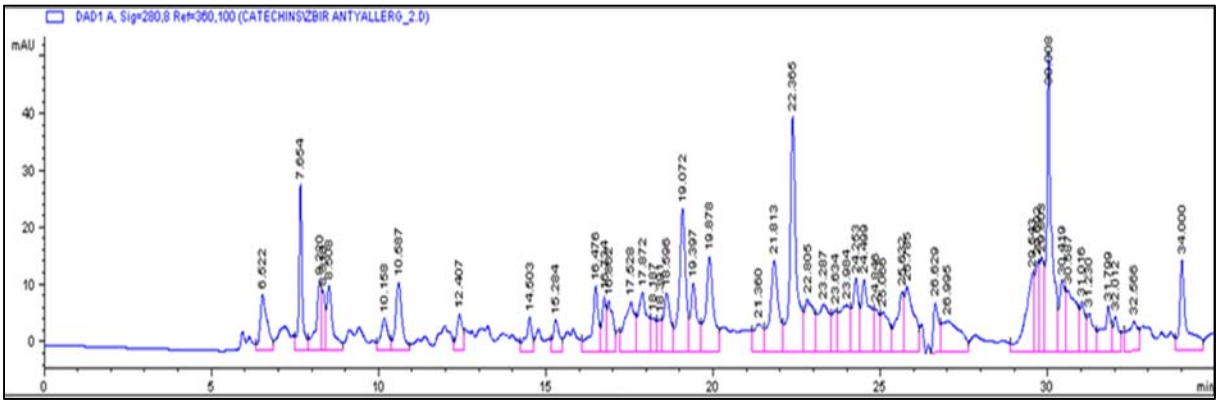

Fig 1: HPLC chromatogram of components of tannins in antidiabetic plant collection (λ = 255 nm).

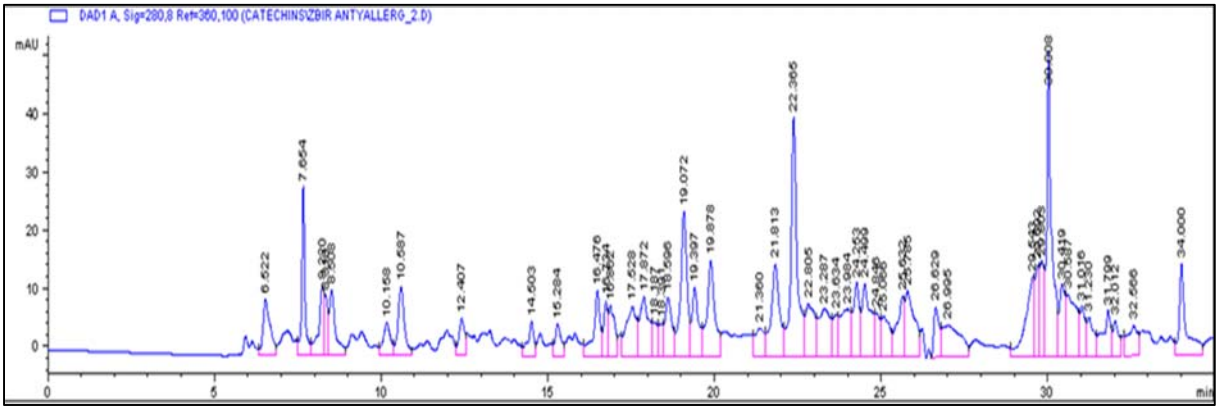

Fig 2: HPLC chromatogram of components of tannins in antidiabetic plant collection (λ = 280 nm).

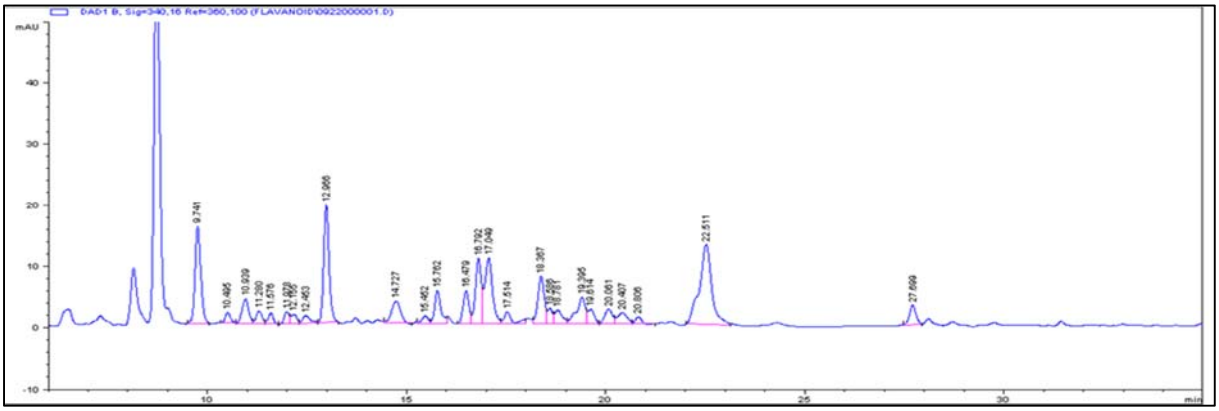

Fig 3: HPLC chromatogram of components of flavonoids in antidiabetic plant collection (λ = 340 nm).

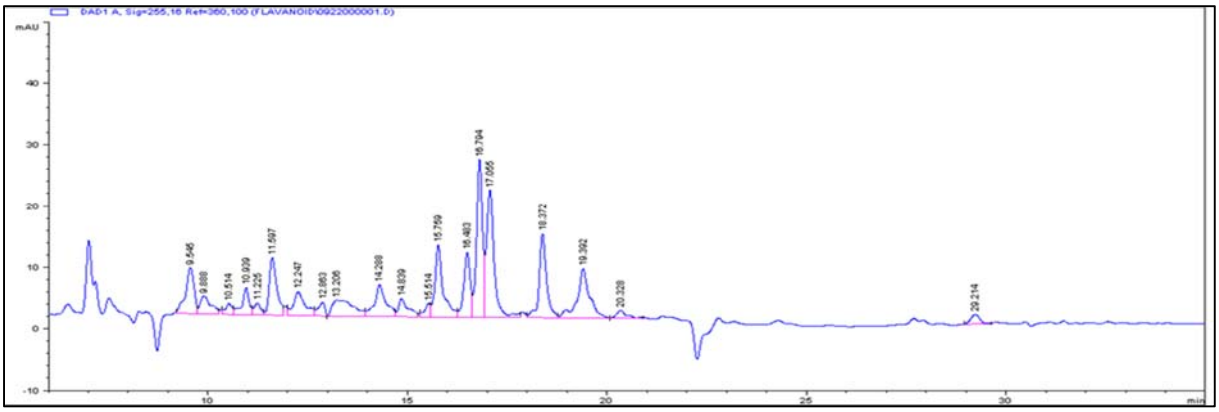

Fig 4: HPLC chromatogram of components of flavonoids and coumarin in antidiabetic plant collection (λ = 255 nm).

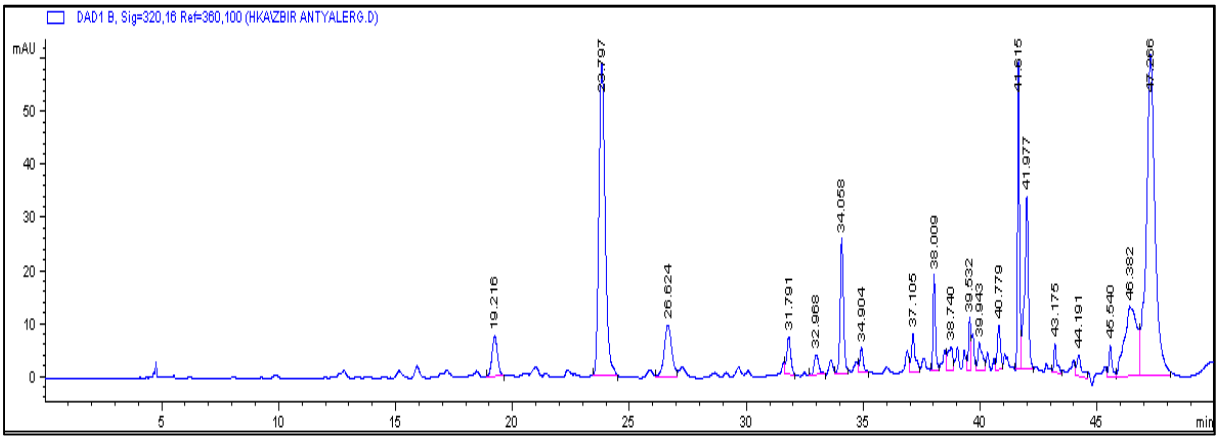

Fig 5: HPLC chromatogram of components of hydroxycinnamic acids in antidiabetic plant collection (λ = 320 nm).

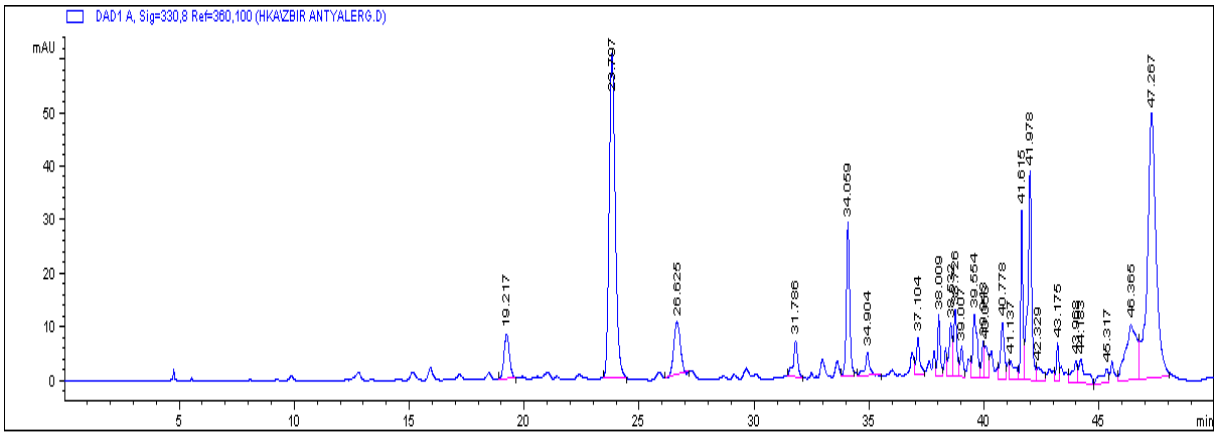

Fig 6: HPLC chromatogram of components of hydroxycinnamic acids in antidiabetic plant collection (λ = 330 nm).

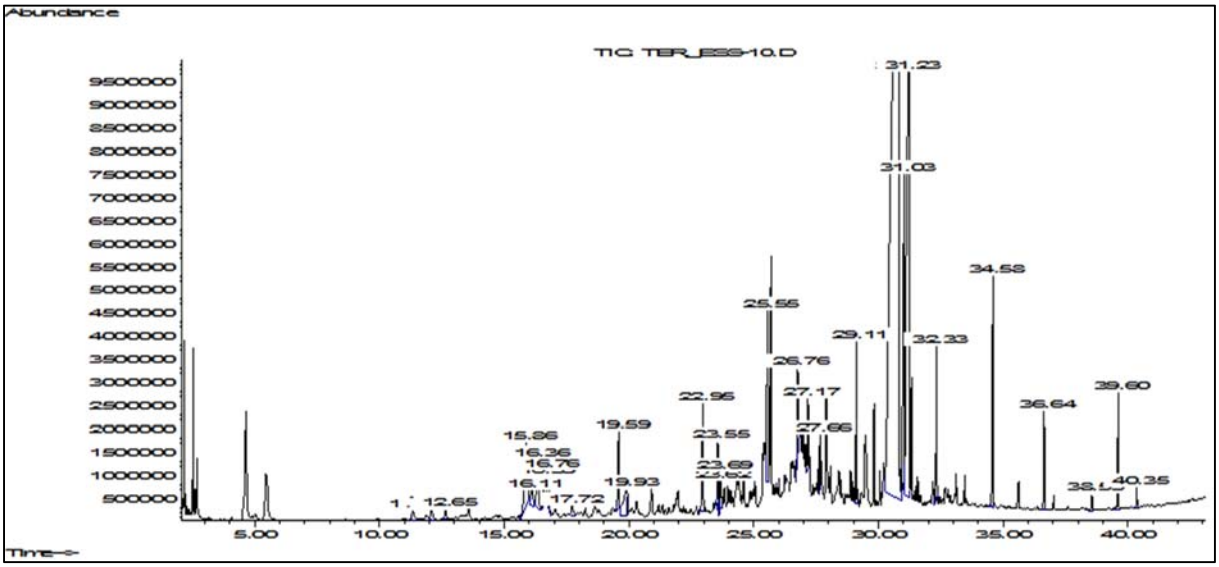

Fig 7: Chromatogram of components of essential oil in antidiabetic plant collection.

Essential oils have a wide range of therapeutic effects that gives them the opportunity to occupy a significant place in the arsenal of therapeutic and preventive tools of modern medicine [19]. The most characteristic pharmacological effects of essential oils are analgesic, anti-inflammatory, antimicrobial, antiviral, antitoxic, antioxidant activity and the impact on the secretory-motor function of the liver and gastrointestinal tract, brain function, circulation, etc. [20, 21].

Exploring the qualitative and quantitative composition of essential oil in antidiabetic plant collection was identified 29 components (Fig. 7).

The main components of essential oil in antidiabetic plant collection are alantolaktone – 2.85% isoalantolactone – 0.83%. cariophilenoxyde – 0.14%. tricosane – 0.14%. trans-epoxy linalool – 0.11% (Table. 3).

Table 2: Individual content of phenolic compounds in antidiabetic plant collection (HPLC, %)

Name of substances	Contents, %
Hydroxycinnamic acids	
Rosemary acid	0.02
p-coumarin acid	0.02
Ferulic acid	0.02
Chlorogenic acid	0.2
Coffee acid	0.03
Flavonoids	
Apigenin	0.002
Quercetin	0.007
Hyperoside	0.06
Luteolin	0.006
Isoquercetin	0.1
Rutin	0.06
Coumarins	
Coumarin	0.005
Components of tannins	
Gallic acid	0.04
Galocatechine	0.21
Epigalocatechine	1.64
Catechine	0.1
Epicatechine	0.22
Catechine galat	0.07
Epicatechine galat	0.17
Ellagic acid	0.005

Table 3: The composition of essential oil in antidiabetic plant collection

№	Retention time	The name of the composition of essential oil	Contents, %
1.	11.32	Phenilacetaldehyde	0.016
2.	12.1	Trans-linalooloxyde	0.017
3.	12.65	Cis-linalooloxyde	0.011
4.	15.85	Trans-epoxylinaloole	0.111
5.	16.11	Cis-epoxylinaloole	0.023
6.	16.36	para-cymen-8-ol	0.066
7.	16.59	Isopara-cymen-8-ol	0.031
8.	16.76	α-terpineol	0.042
9.	17.72	3,5-nonadien-7-in-2-ol	0.013
11.	19.93	Nonane acid	0.066
12.	22.95	Heranilacetone	0.068
13.	23.55	Heranilpropionate	0.041
14.	23.61	β-ionone	0.021
15.	23.69	β-ionon-5.6-epoxide	0.020
16.	25.55	Cariophilenoxyde	0.138
17.	26.75	β-evdesmole	0.066
18.	27.17	α-bisabolole	0.038
19.	27.65	Dyisopropylnaphthalene	0.033
20.	29.11	Hexahydropharnesylacetone	0.082
21.	30.83	Alantolaktone	2.848
22.	31.03	Alantolaktone (close isomer)	0.272
23.	31.23	Isoalantolactone	0.829
24.	32.32	Heneykosane	0.078
25.	34.58	Tricosane	0.137
26.	36.63	Pentacosane	0.048
27.	38.54	Heptacosane	0.012
28.	39.6	Squalene	0.064
29.	40.34	Nonacosane	0.011

4. Conclusions

1. Phytochemical analysis of antidiabetic plant collection was done and was established the presence of such substances of secondary synthesis as flavonoids, hydroxycinnamic acids, tannins, coumarins, essential oils.
2. Flavonoids–rutin, quercetin, kaempferol, hyperoside, apigenin; hydroxycinnamic acid – chlorogenic, coffee, ferulic and p-cumarin acids were identified in antidiabetic plant collection by TLC method.
3. It was established the quantitative content of phenolic compounds in antidiabetic plant collection by spectrophotometry: flavonoids–2.30%, hydroxycinnamic acids–3.10%. By permanganometry found that quantitative tannins – 6.86%.
4. By HPLC identified and established quantitative content of catechins (galocatechine, epigalocatechine, catechine, epicatechine, catechine galat, epicatechine galat); free gallic and ellagic acids; hydroxycinnamic acids (chlorogenic, rosemary, coffee, ferulic and p-cumarin acid); flavonoids (apigenin, rutin, luteolin, hyperoside, isoquercetine, quercetine) and coumarin.
5. It was studied out the qualitative and quantitative contents of essential oil in antidiabetic plant collection and the main components of them are alantolaktone, isoalantolactone, cariophilenoxyde, tricosane, trans-epoxy linalool.
6. The results of research and experience of traditional medicine and homeopathy are the evidence of advisability of studying bioactive composition and conducting toxicological and pharmacological analysis of antidiabetic plant collection to be used for the treatment of diabetes type II and its complications.

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